Model Experiments pertaining to the Mechanism of Action of Vitamin B_{12}-dependent α-Methyleneglutarate Mutase

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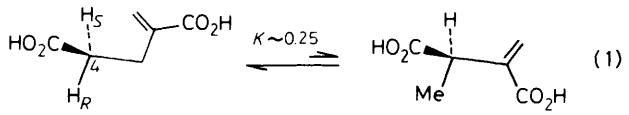
Photolysis of di-t-butyl 1-bromomethylcyclopropane-1,2-dicarboxylate (**1a**) in cyclopropane containing triethylsilane and di-t-butyl peroxide gave the 4-methylenepent-2-yl-1,5-dioic acid radical (**3c**), which was also produced from treatment of (**1a**), its *cis*-isomer (**2b**), di-t-butyl-2-bromo-4-methyleneglutarate (**3a**), or di-t-butyl 2-bromomethyl-3-methylenesuccinate (**4b**), with triphenyltin hydride; these experiments support a mechanism *via* protein-bound free radicals for the B_{12}-dependent enzyme α-methyleneglutarate mutase.

The B_{12}-dependent enzyme α-methyleneglutarate mutase catalyses the molecular rearrangement shown in equation (1), in which H_R is specifically removed from α-methyleneglutarate and the transfer of the acrylate moiety occurs with inversion of configuration at C-4 of α-methyleneglutarate.[1,2] This is one of more than ten enzyme-catalysed rearrangements dependent on the B_{12} coenzyme adenosylcobalamin (AdoCbl).[3] Of the many mechanisms put forward for these reactions, that currently favoured postulates the intermediacy of protein-bound free radicals.[4] For the reaction shown in equation (1) it is necessary to propose at least three intermediate radicals, as shown in Scheme 1.[5,6] We now describe the attempted generation, characterisation, and interconversion of these radicals (as di-t-butyl esters) in model systems for the real enzymic reaction.

Reaction of t-butyl 2-bromomethylacrylate with t-butyl diazoacetate (each reactant 0.45 M in toluene, 80 °C/3 h) gave a 9 : 7 mixture of the bromides (**1a**) and (**2a**), easily separable by silica chromatography (elution with ether–petrol, 1 : 25). The structure of (**1a**) was proved by crystal structure analysis of the corresponding iodide[7] and by its conversion (in formic acid, 4 h/room temp.) into the known[6b] dicarboxylic acid (**1b**).

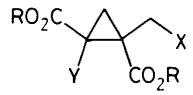


α-methyleneglutarate 3-methylitaconate (1)

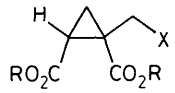
Treatment of t-butyl 2-bromomethylacrylate (0.75 M) in dimethylformamide with t-butyl bromoacetate containing NaH (1 equiv.) at 0 °C (2 h) → room temp. (30 min), gave di-t-butyl 2-bromo-4-methyleneglutarate (**3a**) [55% after silica chromatography, elution with dichloromethane–petrol, 1 : 4]. The carbanion from reaction of di-t-butyl citraconate (0.2 M) in tetrahydrofuran with lithium di-isopropylamide (1 equiv.) at −78 °C was trapped by methanal (an excess of gaseous CH_2O was bubbled into reaction mixture over 2 h) to give di-t-butyl 2-hydroxymethyl-3-methylenesuccinate (**4a**) [39% after silica chromatography, elution with ethanol–ethyl acetate–petrol, 0.5 : 1 : 10]. The alcohol (**4a**) was converted into the bromide (**4b**) by treatment of a 1.0 M solution of (**4a**) in dimethylformamide with triphenylphosphine (1 equiv.) and bromine (1 equiv.) [*n.b.* addition of alcohol (**4a**) to $Ph_3P{\cdot}Br_2$, room temp./overnight]. The bromide (**4b**) was obtained in 41% yield after silica chromatography (elution with dichloromethane–petrol, 1 : 2).

The bromides (2.3×10^{-3} M in benzene) containing a trace of azobisisobutyronitrile) were treated with Ph_3SnH† (10^{-2} M)

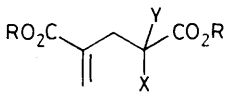
† The use of Ph_3SnH rather than Bu_3SnH (*cf.* Tada *et al.*[8]) was preferred in our studies because it enabled us to exclude more readily the formation of products derived from addition of the tin hydride to the double bond of (**3a**) or (**3b**).



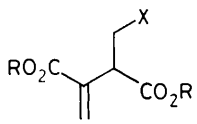
(**1a**); R = $C(Me)_3$, Y = H, X = Br
(**1b**); R = Y = H, X = Br
(**1c**); R = $C(Me)_3$, Y = H, X = free electron
(**1d**); R = $C(Me)_3$, Y = D, X = Br



(**2a**); R = $C(Me)_3$, X = Br
(**2b**); R = $C(Me)_3$, X = free electron

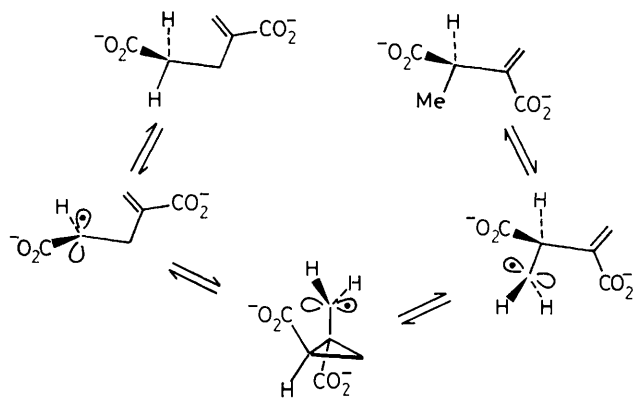


(**3a**); R = $C(Me)_3$, Y = H, X = Br
(**3b**); R = $C(Me)_3$, Y = X = H
(**3c**); R = $C(Me)_3$, Y = H, X = free electron
(**3d**); R = $C(Me)_3$, Y = D, X = free electron
(**3e**); R = $C(Me)_3$, Y = H, X = $Co(dmgH)_2py$



(**4a**); R = $C(Me)_3$, X = OH
(**4b**); R = $C(Me)_3$, X = Br
(**4c**); R = $C(Me)_3$, X = H
(**4d**); R = $C(Me)_3$, X = free electron
(**4e**); R = $C(Me)_3$, X = $Co(dmgH)_2py$

dmgH = dimethylglyoxime monoanion, py = pyridine.



Scheme 1. Pathway for α-methyleneglutarate mutase *via* free radicals [*n.b.* the abstraction of a hydrogen atom from a substrate molecule is achieved by the adenosyl radical derived from homolysis of the Co–C σ-bond of AdoCbl (see refs. 3 and 4)].

for 80—200 min at 50 °C.[8] Compounds (**1a**), (**2a**), and (**3a**) gave di-t-butyl α-methyleneglutarate (**3b**) as the exclusive product. However, compound (**4b**) gave a 2 : 1 mixture of (**3b**) and (**4c**) [the latter was not detected (≤2%) in the reaction mixtures derived from (**1a**), (**2a**), and (**3a**)]. We suggest that reaction of (**4b**) with Ph_3SnH gives radical (**4d**), which is either quenched by Ph_3SnH or rearranges *via* (**1c**) or (**2b**) into radical (**3c**), which is quenched by Ph_3SnH. Obviously, starting from (**1a**) gives (**1c**) directly, which ring opens to (**3c**) faster than it can be quenched by Ph_3SnH. Similarly, (**2a**) → (**2b**) → (**3c**), whilst (**3a**) yields (**3c**) directly.

Photolysis at −112 °C of bromide (**1a**) in cyclopropane containing triethylsilane and di-t-butyl peroxide, in the cavity of an e.s.r. spectrometer,[5] generated an e.s.r. spectrum consistent with the formation of radical (**3c**): *g* 2.0032(5), *a*(2H) 21.37 G, *a*(1H) 20.37 G, *a*(1H) 1.6 G, *a*(1H) 0.75 G (G = 10^{-4} T). Confirmation of this assignment was obtained by analogous photolysis of the deuteriobromide (**1d**) [prepared from t-butyl 2-deuteriodiazoacetate and t-butyl 2-bromomethylacrylate in a similar manner to (**1a**)], which led to radical (**3d**): *g* 2.0034(9), *a*(2H) 21.5 G, *a*(D) 3.125 G, *a*(1H) 1.125 G, *a*(1H) 0.375 G (note the reduction of one of the coupling constants by a factor of 6.51). Curiously, similar photolytic experiments with bromides (**3a**) and (**4b**) did not generate radical (**3c**) [or (**4d**) from (**4b**)]. The nature of the radicals formed in these trials is subject to further investigation.

The experiments described have spectroscopically characterised radical (**3c**) and have shown its derivation from radical (**4d**) *via* (**1c**) or (**2b**). They provide a model system to support the postulate that the enzymic reaction of equation 1 proceeds *via* protein-bound free radicals (as in Scheme 1). We believe that alternative mechanisms *via* organocobalt intermediates are less plausible, especially as the alkylcobaloximes (**3e**) and (**4e**) derived from bromides (**3a**) and (**4b**), respectively, are relatively stable.[9] For the enzymic reaction it can be inferred that the cyclopropylcarbinyl radical shown (Scheme 1) is the intermediate connecting the two but-3-enyl radicals. This is because the *trans*-isomer, but not the *cis*-isomer, of 1-methylcyclopropane-1,2-dicarboxylic acid is a competitive inhibitor of α-methyleneglutarate mutase.[1] From the evidence presented above it is not possible to determine the relative merits of (**1c**) and (**2b**) as intermediates between (**3c**) and (**4d**). Furthermore, the equilibrium and rate constants involving the protein-bound but-3-enyl radicals (Scheme 1) are such as to enable thermodynamic equilibrium to be attained between α-methyleneglutarate and 3-methylitaconate, equation (1). However, in the model experiments described, the equilibrium and rate constants are such as to preclude detection of radical (**4d**) as a product of the ring opening of either (**1c**) or

(**2b**), and it is not formed in significant amount when bromide (**3a**) is reacted with Ph_3SnH; no (**4c**) was seen.

Received, 23rd May 1989; Com. 9/02185J

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